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Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons

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Abstract: **BACKGROUND:** Methicillin-resistant coagulase-negative staphylococci (MR-CNS) are of increasing importance to animal and public health. In veterinary medicine and along the meat and milk production line, only limited data were so far available on MR-CNS characteristics. The aim of the present study was to evaluate the prevalence of MR-CNS, to identify the detected staphylococci to species level, and to assess the antibiotic resistance profiles of isolated MR-CNS strains. **RESULTS:** After two-step enrichment and growth on chromogenic agar, MR-CNS were detected in 48.2% of samples from livestock and chicken carcasses, 46.4% of samples from bulk tank milk and minced meat, and 49.3% of human samples. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), 414 selected MR-CNS strains belonged to seven different species (*S. sciuri*, 32.6%; *S. fleurettii*, 25.1%; *S. haemolyticus*, 17.4%; *S. epidermidis*, 14.5%, *S. lentus*, 9.2%; *S. warneri*, 0.7%; *S. cohnii*, 0.5%). *S. sciuri* and *S. fleurettii* thereby predominated in livestock, BTM and minced meat samples, whereas *S. epidermidis* and *S. haemolyticus* predominated in human samples. In addition to beta-lactam resistance, 33-49% of all 414 strains were resistant to certain non-beta-lactam antibiotics (ciproflaxacin, clindamycin, erythromycin, tetracycline). **CONCLUSIONS:** A high prevalence of MR-CNS was found in livestock production. This is of concern in view of potential spread of *mecA* to *S. aureus* (MRSA). Multiresistant CNS strains might become an emerging problem for veterinary medicine. For species identification of MR-CNS isolated from different origins, MALDI-TOF MS proved to be a fast and reliable tool and is suitable for screening of large sample amounts.

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RESEARCH ARTICLE

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Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons

Helen Huber¹, Dominik Ziegler², Valentin Pflüger², Guido Vogel², Claudio Zweifel¹, Roger Stephan^{1*}

Abstract

Background: Methicillin-resistant coagulase-negative staphylococci (MR-CNS) are of increasing importance to animal and public health. In veterinary medicine and along the meat and milk production line, only limited data were so far available on MR-CNS characteristics. The aim of the present study was to evaluate the prevalence of MR-CNS, to identify the detected staphylococci to species level, and to assess the antibiotic resistance profiles of isolated MR-CNS strains.

Results: After two-step enrichment and growth on chromogenic agar, MR-CNS were detected in 48.2% of samples from livestock and chicken carcasses, 46.4% of samples from bulk tank milk and minced meat, and 49.3% of human samples. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), 414 selected MR-CNS strains belonged to seven different species (*S. sciuri*, 32.6%; *S. fleurettii*, 25.1%; *S. haemolyticus*, 17.4%; *S. epidermidis*, 14.5%; *S. lentus*, 9.2%; *S. warneri*, 0.7%; *S. cohnii*, 0.5%). *S. sciuri* and *S. fleurettii* thereby predominated in livestock, BTM and minced meat samples, whereas *S. epidermidis* and *S. haemolyticus* predominated in human samples. In addition to beta-lactam resistance, 33-49% of all 414 strains were resistant to certain non-beta-lactam antibiotics (ciprofloxacin, clindamycin, erythromycin, tetracycline).

Conclusions: A high prevalence of MR-CNS was found in livestock production. This is of concern in view of potential spread of *mecA* to *S. aureus* (MRSA). Multiresistant CNS strains might become an emerging problem for veterinary medicine. For species identification of MR-CNS isolated from different origins, MALDI-TOF MS proved to be a fast and reliable tool and is suitable for screening of large sample amounts.

Background

It is assumed that methicillin-resistance genes had evolved in coagulase-negative staphylococci (CNS) and were then horizontally transferred among staphylococci [1,2]. Particularly *S. sciuri* [3] and *S. fleurettii* [4] are discussed as natural reservoir of the methicillin-resistance gene *mecA*. The *mecA* gene is located on a mobile genetic element called staphylococcal cassette chromosome (SCC) and confers resistance to methicillin by encoding an altered penicillin-binding protein (PBP2 α), which shows limited affinity to beta-lactam antibiotics.

The *mecA* gene is now distributed among both coagulase-positive and -negative staphylococcal species.

In recent years, the isolation of methicillin-resistant CNS (MR-CNS) from diverse sources was reported. Dakic et al. [5] detected MR-CNS on medical devices, Silva et al. [6] in healthy humans, de Mattos et al. [7] and Ruppe et al. [8] in ambulatory patients, and Diekema et al. [9] in human bloodstream infections. According to Kloos and Bannerman [10], *S. epidermidis* and *S. haemolyticus* are the principal pathogens involved in foreign body infections. Miragaia et al. [11] discussed *S. epidermidis* as one of the most important causes for hospital-acquired bacteremia and found many isolates from hospitalized patients to be methicillin-resistant. Recently, several authors reported MR-CNS also in

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healthy animals. The existence of MR-CNS in animals was first described by Kawano et al. [12], who detected MR-CNS in chicken. Worldwide, MR-CNS were isolated from horses [13-15], dogs [15], cattle [16,17], as well as from sheep, goats, and pigs [17]. However, MR-CNS were also found in animals with clinical infections. Van Duijkeren et al. [18] detected methicillin-resistant *S. haemolyticus* from cats with cystitis and rhinitis, from dogs with bronchitis and pyoderma, and from a horse with vaginitis. Moreover, Fessler et al. [19] reported different MR-CNS species in association with bovine mastitis.

However, comprehensive data on MR-CNS along the meat and milk production line were so far missing. The present work is a subsequent study of a previously published study on the prevalence of MRSA in Switzerland. The aim of the present study was to evaluate the occurrence of MR-CNS in livestock (cows, calves, pigs) and chicken carcasses, bulk tank milk (BTM) and minced meat, as well as people in contact with farm animals (veterinarians, pig farmers, and employees of a chicken and a pig and cattle abattoir), to identify the occurring CNS species, and to assess antibiotic resistance profiles of isolated strains.

Results

MR-CNS occurrence

Using the described procedure, MR-CNS were detected in 1'013 (48.3%) of the 2'099 analyzed samples from livestock and chicken carcasses, BTM and minced meat, and humans (Table 1). Amongst the 1'428 samples from livestock and chicken carcasses, MR-CNS were found in 48.2% of samples. Thereby, the proportion of positive samples ranged from 36.3% in pigs to 72.0% in calves. Analysis of the 211 samples from BTM and minced meat yielded

an average MR-CNS occurrence of 46.4% (32.4% in meat and 62.0% in BTM). Of the 460 human samples, 49.3% turned out to test positive for MR-CNS (from 26.3% in slaughterhouse employees to 67.6% in pig farmers).

Species identification

A total of 414 strains were selected and subjected to species identification using MALDI-TOF MS. Amongst samples, seven different CNS species were detected (Table 2). Sequencing of *sodA* from a subset of 25 strains (5 *S. sciuri*; 5 *S. fleurettii*; 5 *S. haemolyticus*; 5 *S. epidermidis*; 3 *S. lentus*; 1 *S. cohnii*; 1 *S. warneri*) confirmed the results obtained by MALDI-TOF MS. Overall, *S. sciuri* (32.6%) and *S. fleurettii* (25.1%) were most frequently found, followed by *S. haemolyticus* (17.4%), *S. epidermidis* (14.5%), and *S. lentus* (9.2%). Moreover, *S. cohnii* and *S. warneri* were only rarely identified (<1.0%).

Within the 191 selected MR-CNS strains of animal origin, the majority of strains was identified as *S. sciuri* (63.4% of strains), followed by *S. fleurettii* (17.8%), *S. lentus* (15.7%), *S. haemolyticus* (1.6%), *S. epidermidis* (1.0%), and *S. warneri* (0.5%). Thirty of the 38 *S. lentus* strains isolated originated from chicken. Of the 51 strains isolated from calves, 50 were identified as *S. sciuri*. The 52 strains from pigs were identified as *S. sciuri* (55.8%) and *S. fleurettii* (44.2%). The 50 MR-CNS strains isolated from cows also mainly consisted of *S. sciuri* (72.0%) and *S. fleurettii* (22.0%).

Of the 84 MR-CNS strains isolated from BTM and minced meat samples, 76.2% of strains were identified as *S. fleurettii*, followed by 15.5% of strains identified as *S. sciuri*. The remaining seven strains belonged to *S. haemolyticus* (n = 3), *S. epidermidis* (n = 2), *S. cohnii* (n = 1), and *S. warneri* (n = 1).

Table 1 Prevalence of MR-CNS strains detected in samples from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons

Origin	No. of sampled animals, humans and products	No. of MR-CNS positive samples (%)	No. of MR-CNS isolates
Pigs (from 241 farms) ¹⁾	716 ³⁾	260 (36.3)	260
Cows (from 253 farms) ¹⁾	340 ⁴⁾	177 (52.1)	180
Calves (from 187 farms) ¹⁾	300 ⁴⁾	216 (72.0)	218
Chicken (from 72 farms) ²⁾	72	35 (48.6)	38
Bulk tank milk (from 100 farms)	100	62 (62.0)	62
Minced meat	111	36 (32.4)	36
Veterinarians ¹⁾	133	80 (60.2)	84
Pig farmers ¹⁾	148	100 (67.6)	105
Slaughterhouse employees ¹⁾	179	47 (26.3)	48
Total	2099	1013 (48.3)	1031

1) Nasal swabs.

2) Herd-wise pooled neck skin samples.

3) At most three animals per farm were sampled.

4) At most two animals per farm were sampled.

Table 2 Species distribution of MR-CNS strains from different origins

Origin	No. of strains	Species distribution						
		<i>S. sciuri</i>	<i>S. fleurettii</i>	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>S. lentus</i>	<i>S. warneri</i>	<i>S. cohnii</i>
Pigs	52	29	23	0	0	0	0	0
Cows	50	36	11	2	0	0	1	0
Calves	51	50	0	1	0	0	0	0
Poultry	38	6	0	0	2	30	0	0
BTM	50	11	37	1	0	0	0	1
Minced meat	34	2	27	2	2	0	1	0
Veterinarians	50	0	1	33	14	0	1	1
Pig farmers	50	0	2	23	25	0	0	0
SH	39	1	3	10	17	8	0	0
Total	414	135	104	72	60	38	3	2

BTM: bulk tank milk; SH: Slaughterhouse employees.

Of the 139 MR-CNS strains isolated from humans, *S. haemolyticus* (47.5%) and *S. epidermidis* (40.3%) accounted for the great majority. *S. lentus* were only isolated from eight employees of a poultry slaughterhouse, whereas *S. fleurettii* was found in a veterinarian, two pig farmers, and three slaughterhouse employees. The remaining three MR-CNS strains isolated from two veterinarians and a slaughterhouse employee were identified as *S. cohnii*, *S. warneri* and *S. sciuri*.

Antimicrobial susceptibility testing

The results of the phenotypic antibiotic resistance testing of the 414 MR-CNS strains are shown in Tables 3, 4 and 5. Although all characterized strains harbored the *mecA* gene, phenotypic resistance to beta-lactam antibiotics (ampicillin, cefoxitin, oxacillin, penicillin) varied between 63.5% (cefoxitin) and 97.8% (oxacillin). Resistance to ciprofloxacin, clindamycin, erythromycin, gentamicin, sulphamethoxazole/trimethoprim, and tetracycline varied between 14.7% and 49.0%. On the other hand, resistance to rifampin and vancomycin was only detected in 0.7% and 0.2% of strains, respectively. Table 5 shows the species-specific situation of MR-CNS strains to cefoxitin.

With regard to resistance rates of individual MR-CNS species (Table 3), more than 87% of the *S. sciuri* and *S. haemolyticus* strains showed phenotypical resistance to beta-lactam antibiotics. On the other hand, of the *S. epidermidis*, *S. fleurettii*, and *S. lentus* strains only 60% to 80% were resistant to beta-lactam antibiotics. Apart from one exception, more than 54% of strains from cows, calves, veterinarians and pig farmers were resistant to ciprofloxacin, clindamycin, erythromycin, and tetracycline (Table 4). In contrast, apart from two exceptions, less than 27% of strains isolated from BTM, minced meat, pigs and slaughterhouse employees, were resistant to the four antibiotics mentioned above. No strains from BTM, minced meat, and chicken were resistant to sulphamethoxazole/trimethoprim (SxT), whereas 40.0% of strains from pig farmers and 64.0% of strains from veterinarians showed resistance to SxT. None of the strains isolated from BTM and minced meat samples were resistant to rifampin or vancomycin.

Discussion

Methicillin-resistant staphylococci are a major concern to public and animal health. In veterinary medicine and along the meat and milk production line only very

Table 3 Species distribution and antibiotic resistance of the 414 MR-CNS strains

Species	No. of strains	Percentage of resistant strains											
		Oxa	Pen	Amp	Cef	Ery	Tet	Cli	Cip	SxT	Gen	Rif	Van
<i>S. sciuri</i>	135	100	97.8	97.8	83.7	66.7	77.8	65.2	40.7	11.9	19.3	2.2	0
<i>S. fleurettii</i>	104	100	65.4	66.3	32.7	5.8	4.8	18.3	0	0	0	0	1.0
<i>S. haemolyticus</i>	72	90.3	95.8	95.8	87.5	88.9	80.6	65.3	80.6	72.2	31.9	0	0
<i>S. epidermidis</i>	60	96.7	86.7	75.0	46.7	58.3	48.3	25.0	35.0	13.3	20.0	0	0
<i>S. lentus</i>	38	100	78.9	86.8	52.6	18.4	15.8	28.9	2.6	5.3	0	0	0
<i>S. warneri</i>	3	100	100	100	100	0	0	0	0	0	0	0	0
<i>S. cohnii</i>	2	100	100	100	100	50.0	0	50.0	0	0	0	0	0
Total	414	97.8	86.0	85.3	63.5	49.0	49.0	43.7	32.6	18.8	14.7	0.7	0.2

Amp: ampicillin; Cef: cefoxitin; Cip: ciprofloxacin; Cli: clindamycin; Ery: erythromycin; Gen: gentamicin; Oxa: oxacillin; Pen: penicillin; Rif: rifampin; SxT: sulphamethoxazole/trimethoprim; Tet: tetracycline; Van: vancomycin.

Origin	No. of strains	Percentage of resistant strains											
		Oxa	Pen	Amp	Cef	Ery	Tet	Cli	Cip	SxT	Gen	Rif	Van
Pigs	52	100	75.0	76.9	53.8	26.9	44.2	25.0	3.8	15.4	19.2	0	1.9
Cows	50	100	94.0	96.0	90.0	54.0	58.0	58.0	44.0	4.0	8.0	4.0	0
Calves	51	100	96.1	96.1	70.6	88.2	92.2	86.3	62.7	15.7	17.6	0	0
Chicken	38	100	78.9	86.8	68.4	18.4	18.4	18.4	5.3	2.6	2.6	0	0
Bulk tank milk	50	100	76.0	78.0	42.0	10.0	8.0	4.0	6.0	0	2.0	0	0
Minced meat	34	100	73.5	70.6	35.3	8.8	11.8	5.9	8.8	0	2.9	0	0
Veterinarians	50	96.0	94.0	90.0	78.0	80.0	84.0	58.0	64.0	64.0	26.0	0	0
Pig farmers	50	96.0	100	98.0	74.0	72.0	76.0	56.0	62.0	40.0	38.0	0	0
Sh employees	39	87.2	74.4	66.7	38.5	48.7	12.8	23.1	25.6	12.8	5.1	2.6	0
Total	414	97.7	84.7	84.3	61.2	45.2	45.0	37.2	31.4	17.2	13.5	0.7	0.2

limited data were available on characteristics of MR-CNS. The present study provides an overview on the MR-CNS situation in Swiss livestock, chicken carcasses, BTM, minced meat, and persons in contact with farm animals. Since data of studies with comparable procedures are lacking, direct comparison of results is not possible. Therefore, we discussed our results in comparison to data that we considered most appropriate and as close to our own results as possible. In our study, MR-CNS were detected at an average of 48.3% in 2'099 samples. The occurrence varied thereby from 26.3% in slaughterhouse employees to 72.0% in calves. The MR-CNS occurrence rates mentioned above are in contrast to the low MRSA prevalence in Switzerland [20].

is especially relevant, since this CNS species is also reported to cause fatal exudative epidermitis in piglets [21], which is normally caused by exfoliative toxin producing *S. hyicus*. Interestingly, methicillin-resistant *S. sciuri* were also found to predominate in horses [22,23]. Furthermore, the MR-CNS strains isolated from chicken in our study were mainly (78.9%) of *S. lentus*, whereas Kawano et al. [12] did not detect methicillin-resistant *S. lentus* in their samples. Methicillin-resistant *S. lentus* have also been described in other animal species such as horses [14,22,24], pigs, cattle, sheep, and goats [17]. Other data on species-specific identification of MR-CNS from livestock are limited.

In the BTM and minced meat samples of the present study, MR-CNS were detected in 32.4% of minced meat and in 62.0% of BTM samples. Respective data on MR-CNS from these sample categories are hardly found. The species predominantly found in BTM and minced meat samples of the present study, were *S. fleurettii* accounting for 76.2% of all analyzed strains, followed by *S. sciuri* comprising 15.5% of strains. In bovine quarter milk samples, Gillespie et al. [25] found mainly CNS of *S. chromogenes*, *S. hyicus*, *S. epidermidis*, and *S. simulans*. In a subsequent study, Sawant et al. [26] found 11 of 37 *S. epidermidis* strains to harbor the *mecA* gene and show phenotypic resistance to ampicillin

[illegible]

and oxacillin. On the other hand, CNS are also frequently isolated from clinical and in particular subclinical intramammary infections in cattle [27] and small ruminants [28-30]. CNS found in this context generally showed a species distribution different from that present in our samples.

In the 460 nasal swabs from humans in contact with farm animals, MR-CNS were detected in 49.3% of samples. In view of contact persons, two studies found the prevalence of MR-CNS in horse personnel to be 37% and 63% [22,23]. Moreover, a study among healthy humans without association to hospital or animals found an MR-CNS prevalence of 24% [6]. In human samples of the present study, the most frequently isolated species were *S. haemolyticus* (47.5%) and *S. epidermidis* (40.3%). MR-CNS of species *S. haemolyticus* [23] and *S. epidermidis* [22] were also frequently isolated from horse personnel. These two predominant species are also of importance for human infections. As discussed above, *S. epidermidis* and *S. haemolyticus* are frequently isolated CNS species involved in clinical cases of humans.

There are not much data available on the antimicrobial susceptibility of MR-CNS isolated from colonized livestock and humans, or from BTM and minced meat. In the present study, MR-CNS strains from BTM, minced meat, and chicken were only in limited numbers resistant to antibiotics other than beta-lactams. In view of MR-CNS as food contaminants and potential vectors of antibiotic resistance, these results suggest a favorable situation. In contrast, strains isolated from calves, cows, pig farmers, and veterinarians showed multi-resistance to a great extent. This situation may also be caused by frequent direct contact of these individuals with antibiotics. Interestingly, resistance rates of strains from pigs and slaughterhouse employees were in-between the groups mentioned above. Furthermore, it is noteworthy that in our MR-CNS strains, phenotypic resistance to oxacillin was by far a better indicator for the presence of the *mecA* gene than resistance to cefoxitin. Interestingly, for indication of *mecA* in MR-CNS using MIC, both cefoxitin and oxacillin are discussed as valuable approaches [31].

Conclusion

In conclusion, the results obtained indicate a high MR-CNS prevalence (on average 48.3%) in samples from livestock, chicken carcasses, BTM and minced meat, as well as persons in contact with livestock. The occurrence thereby ranged from 26.3% in slaughterhouse employees to 72.0% in calves. This is the first study detecting MR-CNS in a large collection of samples taken along the meat and milk production line. For species identification of MR-CNS isolated from different

origins, MALDI TOF MS proved to be a fast and reliable tool and is therefore suitable for screening of large sample amounts. Furthermore, based on the present data, the occurrence of methicillin-resistant staphylococci should be continuously evaluated in surveillance programs within the scope of veterinary public health aspects. Otherwise, further studies are required to investigate the impact of the high MR-CNS prevalence on human and animal health.

Methods

Bacterial strains

From March through September 2009, we collected a total of 2'099 samples from livestock and chicken carcasses, BTM and minced meat, as well as humans in contact with farm animals. Livestock was sampled at slaughter. Thereby, swabs were collected from the nasal cavity of pigs ($n = 716$), calves ($n = 300$), cows ($n = 340$). From chicken carcasses herd-wise pooled neck skin samples ($n = 72$) were collected, each herd originating from a different farm. Sampled animals (including chicken) originated from more than 800 farms in 10 different cantons distributed throughout Switzerland. Furthermore, 100 BTM and 111 minced meat (pork and beef) samples were tested. Moreover, we analyzed nasal swabs from 148 pig farmers attending two meetings on swine breeding, 133 veterinarians participating in a course on castration of piglets, and 179 slaughterhouse employees working in two different abattoirs (a poultry and a pig and cattle slaughterhouse). All human nasal swabs samples were taken by the persons themselves. They gave us their consent that the samples can be used for this study.

For isolation of MR-CNS strains, a two-step enrichment procedure in Mueller-Hinton broth supplemented with 6.5% NaCl (24 h at 37°C) and in phenol red mannitol broth supplemented with 75 mg/L aztreonam and 5 mg/L cefoxitin (24 h at 37°C) was used. Samples were then plated onto Oxoid Brilliance™ MRSA Agar (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37°C. Of presumptive MR-CNS colonies of beige or white color, one colony (and for some samples with different colony morphologies at most two colonies) was picked, gram-stained, and tested for catalase-activity. All gram-positive, catalase-positive cocci grown beige or white on Oxoid Brilliance™ MRSA Agar were considered MR-CNS. Thereafter, a random selection of 414 strains (34 to 52 isolates per sample origin) was confirmed as genetically methicillin-resistant by detection of the *mecA* gene [32] and then further characterized.

Species identification

MR-CNS species identification was performed by matrix-assisted laser desorption ionization-time of flight

mass spectrometry (MALDI-TOF MS). MALDI-TOF MS was recently described as an effective and powerful tool for reliable identification of staphylococci [33] and CNS in particular [34]. Briefly, of the 414 randomly selected strains, one or two colonies of a fresh overnight culture grown on TSA were suspended in 20 µL formic acid (25%). Thereafter, 20 µL of a saturated solution of sinapinic acid (49508, Sigma-Aldrich, Buchs, Switzerland) in 60% acetonitril (154601, Sigma-Aldrich, Buchs, Switzerland), 0.3% trifluoroacetic acid (T6508, Sigma-Aldrich, Buchs, Switzerland) was added. Of this mixture 1 µL was spotted in duplicates on a steel target plate and air dried at room temperature. Spectra were acquired using a AximaTM confidence (Shimadzu-Biotech Corp., Kyoto, Japan) MALDI-TOF massspectrometer, in the linear positive mode, at a laser frequency of 50 Hz and within a mass range from 2000-30000 Da. Acceleration voltage was 20 kV, and the extraction delay time was 200 ns. A minimum of 10 lasershots per sample was used to generate each ion spectrum. For each bacterial sample, a total of 100 ion spectra were averaged and processed using the LaunchpadTM v.2.8 Software (Shimadzu-Biotech Corp., Kyoto, Japan). Peaks lists were in ASCII format imported into the SARAMISTM (Spectral Archive And Microbial Identification System, AnagnosTec, Potsdam-Golm, Germany) for automated species identification.

For additional confirmation of species identification, 56 randomly chosen strains were identified by sequencing an internal fragment of the *sodA* gene following a protocol of Poyart et al. [35] with slight modifications. DNA sequence-based species identification is currently the most accurate identification method available for CNS [36]. The partial *sodA* gene was amplified by PCR using primers d1 (5'-CCITAYICITAYGAYGCIYTI-GARCC-3') and d2 (5'-ARRTARTAIGCRTGYTCC-CAIACRTC-3'). PCRs were performed in a T3000 thermocycler (Biometra, Goettingen, Germany) in a final volume of 50 µL containing a minimum of 150 ng of DNA, 2 µM of each primer, 200 µM of each deoxynucleoside triphosphate, and 2 U of FastStart High Fidelity polymerase (Roche, Basel, Switzerland) in a 10× reaction buffer (with 18 mM MgCl₂, included in kit). PCR mixtures were denatured (3 min, 95°C) and then subjected to 30 cycles of amplification (60 s of annealing at 39°C, 45 s of elongation at 72°C, and 30 s of denaturation at 95°C). The product was then sequenced and the results were compared to the GenBank database using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Antimicrobial susceptibility testing

To evaluate the phenotypic antibiotic resistance of the selected 414 strains, the disk diffusion method was used in accordance with the guidelines of the Clinical and Laboratory Standards Institute [37] using the following

disks (BD BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD, USA): ampicillin (10 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin (10 units), rifampin (5 µg), sulphamethoxazole/trimethoprim (23.75/1.25 µg), tetracycline (30 µg), and vancomycin (30 µg). Results were interpreted according to the CLSI guidelines, whereby intermediate results were considered resistant.

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Authors' contributions

HH was responsible for isolation and characterization of strains and drafted the manuscript. DZ, VP, and GV performed MALDI-TOF MS analysis and evaluated respective data. CZ and RS designed the study and edited the manuscript. All authors read, commented on, and approved the final manuscript.

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